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An update on biological advancement of *Jatropha curcas* L.: New insight and challenges

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ABSTRACT

Sustainable production of non-food oleaginous crops is highly desirable towards meeting increasing global demands for energy by supplementing the diminishing supply of fossil fuels without affecting food security. *Jatropha curcas* L., a high yielding oleaginous seed crop (30–48% oil), with a simple propagation system, short gestation period and high adaptability to a wide range of climatic and edaphic conditions, qualifies to be a potential candidate for sustainable biofuel production. The crop has been proposed as part of the solution to the challenges of climate change, energy scarcity and provision of rural income, as it can grow in marginal soils with minimal nutrient content. However, in 2010, this view was challenged due to unpredictable yield patterns of the crop, recorded for plantation in marginal and low nutrient environments. The main reason behind the failure to meet its promise, was the paucity of information of the optimal agricultural practices and nutrient requirements required for large scale cultivation of *J. curcas*. The low productivity of seed in *J. curcas* was identified to result from a combination of variation in seed quality and quantity, high male to female flower ratio, asynchronous flowering, seed toxicity and susceptibility to various biotic and abiotic stresses. With the advancement of new technology and the availability of a whole genome sequence for *J. curcas*, several studies addressed at overcoming the shortcomings of the plant *via* biological approaches have been reported, indicating a renewed promise for this energy crop. This review addresses recent developments in improvement of *J. curcas* from the biological perspective, identifies some remaining gaps in knowledge and provides recommendations for future studies towards economically viable *J. curcas* cultivation.

1. Introduction

Jatropha curcas L., belongs to the Euphorbiaceae family and is known for its multiple beneficial applications from biodiesel production [1] to the herbal medicine [reviewed in 2] and cosmetic industries [reviewed in 3]. *J. curcas* is indigenous to Mexico and Central America and has spread widely throughout the tropics [4]. The plant garnered worldwide attention as an alternative source of sustainable energy mainly because of its non-edible high seed oil content. Each mature plant of *J. curcas* produces an average of 4–5 kg of seeds per year when cultivated under suitable conditions with an extensive productive period of around 30–50 years [5]. In addition to its easy propagation by grafting or seed [6], short gestation period, high adaptability to a wide range of environments and soils [reviewed in 7], ability to sequester carbon in degraded soils [8] and capacity for the bioremediation of heavy metal-contaminated soils [9] makes *J. curcas* a crop of choice for biodiesel production [10] in developing countries. As a non-food crop, capable of growth on land unsuitable for food production, and with good potential to address issues related to climate change, growing energy demand and the need to improve rural income, *J. curcas* was seen as an ideal energy crop. Thus in 2009, initiatives were taken by the governments of India, China and Brazil along with several biofuel companies and NGOs for large scale cultivation of *J. curcas* [11]. Other countries such as Myanmar, Malaysia and Malawi also showed their interest to grow this plant. However, in 2010, questions on the sustainability of Jatropha-based biofuel programs were raised, because of its underperformance in large scale cultivation [12–14]. The main reasons behind the failure of *J. curcas* to meet its promises were inadequacy in field validation and agricultural practices, lack of knowledge on nutritional requirements of the crop [15], high variation in seed quantity and quality, high male to female flower ratio [16], seed toxicity [17] and susceptibility to biotic [18] and abiotic stresses [19].

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Subsequently, research on the biological improvement of *J. curcas* has been reported to address many of the apparent shortcomings of this crop.

Previous reviews have highlighted the multiple applications of *J. curcas* [20–26] such as use for biodiesel production [27–33], the status of *J. curcas* cultivation [34,35], socio-economic and environmental aspects [36–38]; sustainability issues [39], breeding aspects [40–42], tissue culture and transformation [7,41–44] biochemical studies [44], seed toxicity [21,26,45,46], usage of latest technology to analyse metabolic pathways [47] and various limitations of *J. curcas* in field conditions, from which suitable breeding strategies have been proposed for the future utilization of this plant [42,48,49]. In the present review, we examine recent studies conducted to address the limitations of *J. curcas* from the biological aspects of harnessing the knowledge on plant-microbe interactions, gene resources and biotechnological tools. We identify the gaps in biological knowledge and applications that remain for this crop and provide recommendations for future biological research for *J. curcas* towards economic feasibility for bioenergy production.

2. Review of studies using biological approaches to address the limitations of *J. curcas* as a biofuel plant

Some of the major challenges to adopt *J. curcas* as potential feedstock of future sustainable fuel have been summarised in Fig. 1. There are several biological approaches that can be used to address these challenges, as discussed in the following sections:

2.1. Optimising *J. curcas* productivity in Low nutrient soil

Poor productivity in low nutrient soil was reported as a major limitation to *J. curcas*'s success as a biofuel crop [4]. To avoid the competition for land use for food crops, the ideal for biodiesel crops is to be grown in low nutrient or barren soils. Due to high phenotypic plasticity, *J. curcas* can easily grow in such soil, but low nutrient soil reduces seed yield [15]. To improve the seed yield, nitrogen fertilizers were initially tested, which enhanced *J. curcas* growth and productivity [50]. However, emission of reactive N_2O from nitrogen fertilizers traps heat in the atmosphere 310 times more effectively than CO_2 [51] and hence is considered as one of the important contributors to global warming. To enrich the fertility level in barren and degraded land, organic amendments (such as animal manure) were used to evaluate germination and growth patterns of *J. curcas* in a field trial [15]. The field trial showed significant enhancement of *J. curcas* growth and biomass compared to the control plants. A disadvantage of the application of organic amendments was that this attracted humivorous termites, which lead to severe damage of the seedlings [15]. To overcome the termite manifestation, use of pesticides has been recommended [15,52]. However, pesticide use risks encouraging the emergence of resistant pest in addition to environmental toxicity [53]. As an alternative, several groups of researchers investigated the effects of beneficial plant-microbe interactions mediated by arbuscular mycorrhizal fungi (AMF), endophytes and plant growth promoting rhizobacteria (PGPR) in *J. curcas*.

AMF transfer inorganic soil nutrients P, Zn and N to the host plant in exchange for organic carbon [54]. Such symbiotic associations of AMF with crop plants are widely known [reviewed in 55]. AMF diversity in the rhizosphere of *J. curcas* was explored in plantation areas in Thailand [56] and India [57,58] from which AMF were seen to promote *J. curcas* growth under a wide range of field conditions, from low to high nutrient soils including acidic, saline and alkaline conditions. Most common fungi species recorded in such symbiosis belong to the genera Glomus, Acaulospora, Gigaspora and Scutellospora [59,60]. Low nutrient soil (low P availability) supplemented with AMF spores (*Glomus aggregatum*, *G. fasciculatum*, *G. interradices*, and *G. mosseae*) showed growth enhancement of *J. curcas* seedlings compared to non-supplemented soil [61]. Similarly, in a field trial, *J. curcas* inoculated with AMF spores (*Glomus fasciculatum* and *Scutellospora calospora*), showed enhanced plant height, higher branch number and larger canopy area compared to the plants supplied with NPK fertilizer [59]. *J. curcas* inoculated with AMF (*Glomus mosseae, G. microcarpum, G. fasciculatum, G. intraradices*, *Gigaspora margarita,* and *G. heterogama*) isolated from the rhizosphere of castor (another oilseed crop of the Euphorbiaceae family) showed improved plant vigor under salt stress conditions, suggesting a vital role of AMF in plant salinity-tolerance [57]. AMF (Glomus, Scutellospora, Entrophosphora and Gigaspora) inoculation also increased the survival rate of *J. curcas* grown in highly acidic, low nutrient soil [62]. *J. curcas* inoculated with AMF grown in heavy metal contaminated soil (Cu and Pb) was also reported to show reduced translocation of heavy metals from the roots to the aerial plant parts [63]. Hence, AMF can be successfully applied as a component of biofertilizers to counteract problems of nutrient deficiency, salinity and metal toxicity in soil. However, since mycorrhizal species often have specific ecological and edaphic requirements for efficient root colonization and plant growth promoting activity, such as soil pH, temperature and organic matter [reviewed in 64], the best performance of AMF in large-scale plantation, may be obtained by use of species indigenous to the particular edaphic condition.

Endophytes are mostly fungi and bacteria that live within living plant tissue. Several novel and beneficial endophytes identified from *J.*

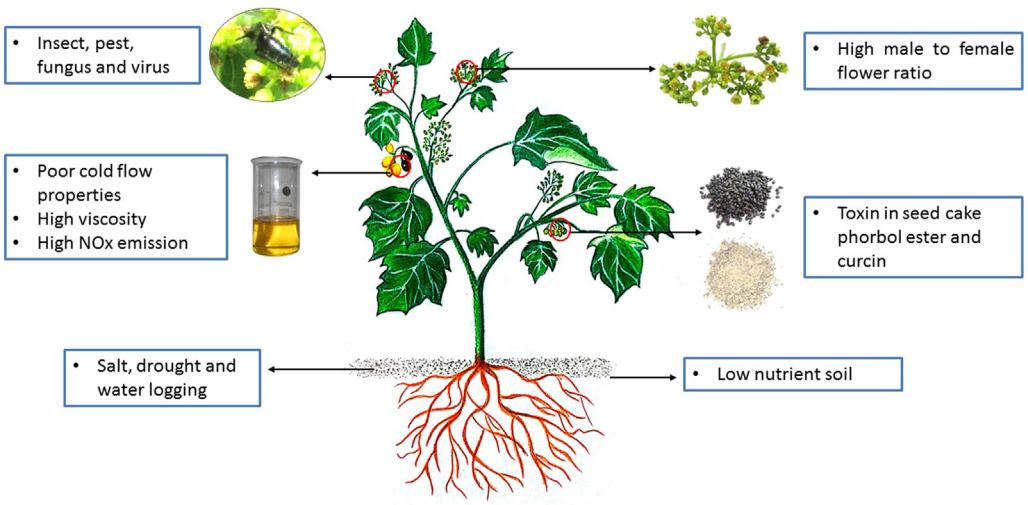

Fig. 1. Limitation of *Jatropha curcas* as biodiesel plant.

Table 1
Plant growth promoting endophytes reported in *J. curcas*.

Endophytes	Plant part	Beneficial features of Endophytes	References
Rhizobacteria	Roots	Promoted root and shoot growth	[65]
Enterobacter sp.	Roots	Stimulated plant growth and increased average seed number and seed weight in *J. curcas*	[66]
Actinobacteria (Streptomyces, Nonomuraea, Micrococcus and Kibdelosporangium sp.)	Roots, leaves, seeds and stems	Increased fresh weight, shoot and root length and leaf area of *J. curcas* seedlings	[67]
Methylobacterium sp.	Leaves	Promoted biomass and seed production in *J. curcas*	[68]
Firmicutes and Alphaproteobacteria (Paenibacillus, Brevibacillus, Sphingomonas and Rhizobium sp.)	Roots	Promoted growth and development of *Zea mays*	[69]

curcas, showed successful colonization in roots and enhanced plant growth and seed yield, even when grown in low nutrient soil [65–69] (see Table 1). Madhaiyan et al. [65] explored endophytic diversities in leaf, stem and root of *J. curcas*. Molecular and functional characterization of endophytes showed them to be species mainly belonging to α, β, γ-Proteobacteria, Actinobacteria, Firmicutes and Methylobacterium [68]. Among the Methylobacteria isolates, Methylobacterium L2–4 strain was characterized in detail and showed high nitrogen-fixing efficiency, and promoted biomass and seed production in *J. curcas*. Mohanty et al. [69] showed that endophytes isolated from *J. curcas* were also able to improve growth performance of other agricultural crops like maize.

In addition to endophytic bacteria, several PGPR have been identified with potential to enhance the growth and productivity of *J. curcas* on nutrient depleted or contaminated soils. Enterobactor sp. isolated from the rhizosphere of *J. curcas* grown in alkaline, wasteland, sodic and saline soils were shown to be efficient phosphate solubilizers and to have auxin production capacity during screening [70,71], however, their effectiveness in field conditions has not yet been demonstrated. A Chromobacterium sp. isolated as a PGRP from the rhizosphere of *J. curcas*, displayed an ability to degrade dichlorobenzene, a ground water pollutant [72]. Some PGPR sp. that have been commonly used for other crops, such as *Enterobacter clocae*, *Pseudomonas pseudoalcaligenes* [73], *Pseudomonas fluorescens, Pseudomonas putida* [74] and Bacillus sp. [73,75] are also able to colonize the rhizosphere of *J. curcas* and promote fast seed germination and plant growth. While there has been advancement in knowledge of beneficial microbes for yield and growth improvement of *J. curcas* and that will potentially address issues of low productivity in low nutrient and marginal soils, further studies are still needed both for field scale evaluation and towards the low-cost mass production and high storage viability of microbial inoculants for sustainable cultivation.

In addition to soil enrichment, use of cultivars with improved seed yield and stress tolerance capacity for large scale plantation can enhance the productivity of *J. curcas* without effecting agricultural land use. Reviews have summarised promising research outcomes from morphological and molecular screening of elite accessions of *J. curcas* [reviewed in 7, 41]. A requisite for genetic improvement through breeding is an availability of sufficient genetic variability. Studies on morphometric traits such as plant growth, architecture, seed yield, seed weight and oil content among *J. curcas* accessions have shown the existence of considerable variability. However, diversity assessment based solely on morphometric traits are not always reliable, as *J. curcas* shows high plasticity. Additional information from studies with molecular markers including random amplified polymorphic DNA (RAPD), inter simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and sequence-tagged sites (STS) suggest low genetic diversity except among accessions collected from central America and Mexico [76]. Thus, accessions identified from central America and Mexico show the best potential for use in breeding programs [77]. Several research groups worldwide viz, Wageningen University and Research Centre, Plant Research International (Netherlands) and Lao Institute of Renewable Energy (Laos), Surfactant and Bio-energy Resource Centre (Indonesia), Agricultural Research Trust (Zimbabwe), National Oilseeds and Vegetable Oils Development Board of India and Indian Institute of Oilseeds Research (India) have collected and maintain *J. curcas* germplasm repository, housing several thousand accessions of *J. curcas* from various locations around the world. Many of the accessions have been screened for seed and oil traits towards support for breeding and development of stable elite varieties [reviewed in 7]. The details of selection strategies and breeding status of *J. curcas* in Brazil were discussed in de Azevedo et al. [78]. However, release of commercial elite cultivars *via* breeding is still in its infancy. As *J. curcas* is a perennial species with several traits of interest (as described in Fig. 1), the procedure of selection *via* conventional breeding is expected to be very slow (approximately 10–14 years) [78]. Several efficient transformation systems [79,80] and genetically modified *J. curcas* with improved oil trait [81], stress tolerance [82,83] have been reported and can be used in crop improvement. As for any crop, application of a combinatorial approach encompassing biotechnological tools and conventional breeding, are pivotal to expedite the development and release of elite cultivars.

2.2. Enhancing seed yield by increase in female flower ratio

One of the major shortcomings of *J. curcas* for biofuel production, is the unpredictable seed yield, with low seed production being a barrier to commercial success. *J. curcas* plants are mainly monoecious with flowers arranged as an axillary paniculate polychasial cymes inflorescence (Fig. 2a), where the female flower is present at the top surrounded by sub branches which mainly produce male flowers (Fig. 2b). Occasionally hermaphrodite flowers are also observed in *J. curcas* [84,85]. *J. curcas* has a complex floral biology, with floral development divided into 12 phases [86]. The female flower develops as a hermaphrodite up to the sixth phase of floral development and thereafter sexual differentiation begins when stamens are aborted and the pistil develops [86]. In each inflorescence, the male to female ratio has been variously reported as 13.4:1 [87], 27: 1 [88] and 30:1 [16]. The variability in male and female flower ratio and relatively low number of female flowers is one of the reasons for variation of seed yield in *J. curcas* [89].

In recent years, several approaches have been explored towards increasing the female flower proportion in *J. curcas* inflorescences. Exogenous application of plant growth regulators (PGRs) such as gibberellin [90], cytokinin [91], paclobutrazol [92–94], auxin [95] and thidiazuron (TDZ) [96] to the inflorescence showed induction of larger numbers of bisexual flowers, increased female flower number and a 5–11-fold increase in *J. curcas* seed yield. These studies indicated great potential for improvement in *J. curcas* seed yield by the application of PGRs *via* significant increase in female flower ratio to increase fruit production and hence seed yield. Nevertheless, the application of PGR directly to flowers is labour intensive and not an economical and practical solution for a large-scale commercial plantation. Manipulation of genetic control of flowering may provide a better and long-term solution. Several key floral regulatory network genes have been identified in *J. curcas*: Li et al. [97] isolated *J. curcas* flowering locus T

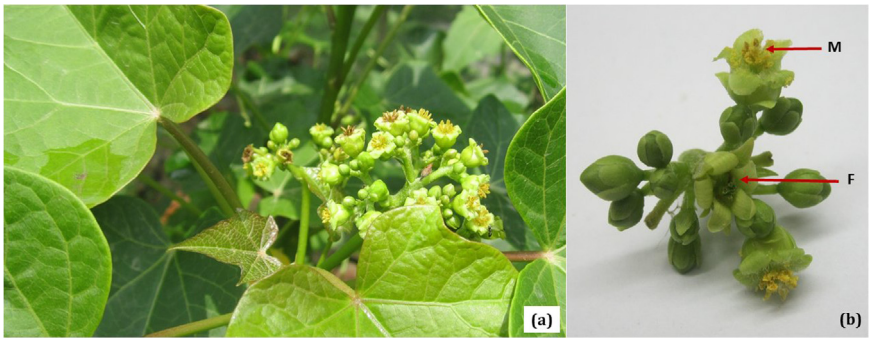

Fig. 2. *Jatropha curcas* inflorescence (a) *J. curcas* plant with flowers arranged in axillary paniculate polychasial cyme inflorescences. (b) Male flowers (M) and female flowers (F).

homolog (*JcFT*), which was observed to be expressed mainly in the female flowers, fruits, and seeds. Over-expression of *JcFT* in *J. curcas* resulted in early flowering transgenic lines supporting its role in inducing flower development [97]. Another flower development associated gene LEAFY (*JcLFY*) isolated from *J. curcas*, was shown to have a critical role in the early stage of flower bud formation, as over-expression induced early flowering, solitary flowers, and terminal flowers in *J. curcas* [98]. From a study comparing relative gene expression in tissues associated with flower development in a superior genotype of Jatropha (with high female flower number) and a genotype with low relative numbers of female flowers, abundant transcripts of *SUP* (Superman), *TAA1* (L-tryptophan–pyruvate aminotransferase 1), *CRY2* (Cryptochrome-2), and *CKX1* (Cytokinin dehydrogenase 1) were associated with increased numbers of female flowers [16]. This was explained based on the role of these genes in arresting the development of stamens [16]. Another study which profiled gene expression of *J. curcas* flower buds at six different developmental stages starting from prior to sex differentiation stages and up to mature female flower formation, provided intensive information on genes involved in carpel and stamen development and maturation of male and female flowers [99]. Among 15 useful sex-related candidate genes, gibberellin-regulated protein 4-like protein, CLAVATA1 and adenosine monophosphate-activated protein kinase were associated with stamen differentiation; auxin response factors 6-like protein, AGAMOUS-like 20, RING-H2 finger protein ATL3J, auxin-induced protein 22D, CLAVATA1, and R2R3-MYB transcription factor were linked to embryo sac development, while cytokinin oxidase, auxin repressed-like protein1-like protein, Gibberellin receptor protein 1 and auxin-induced protein X10A were present both during stamen differentiation and embryo sac development [99]. While further detailed functional validation is still needed, the identification of candidate genes from these types of study provides a good foundation for the future manipulation of floral ratio in *J. curcas*.

In addition to the importance of the female flower ratio in inflorescences, efficient pollination, aided by the establishment of pollination services in large scale plantations, aids to maximize fruit yield in *J. curcas* [reviewed in 100]. The studies on floral structure and pollination ecology of *J. curcas* accessions in Thailand [101], India [87,102–104], Brazil [105], China [106,107], Indonesia [108] and Mexico [109] showed that its flower exhibits protandry (in which male reproductive organs mature before the female ones) which favours xenogamy (cross pollination) over geitonogamy (self-pollination). Fruit production was recorded to be highest in open-pollination and in xenogamy. The pollinators of *J. curcas* mainly belong to the orders Hymenoptera, Lepidotera and Diptera [108]. Studies conducted in different geographical locations showed honey bees (*Apis florea*, *A. dorsata*, *A. mellifera*, *Scaptotrigona mexicana* and *Trigona angustula*) [102,103,108–110] to be the most efficient pollinators compared to other insect visitors. This information can be strategically used to increase seed yield by establishment of honey bee hubs along with native pollinators in large scale plantation.

2.3. Detoxification of *J. curcas* seed cake using microbes

J. curcas seed cake is a by-product of oil extraction with high nutritional content, but also containing several non-desired compounds. The seed cakes have higher nutritional quality than soybean meal [111] with high levels of protein (45.3–58.6%), dietary fibre (5–5.5%) and with a gross energy value of 19–48% [112]. Essential amino acid profiling of *J. curcas* seed cake showed higher levels of valine, isoleucine, leucine, histidine and threonine than the recommended dosage for children by FAO/WHO [112]. The rich nutrient content would be ideal for human and animal nutrition, as fertilizer and as a substrate for production of industrial enzymes such as protease and lipase, as it supports good bacterial growth [113–115]. However, the presence of toxins such as phorbol esters, curcin and antinutritional compounds such as phytic acid, tannins and saponin make the seed cake not suitable as food or animal feed currently [112,116]. The use of *J. curcas* seed cake as a biofertilizer is also limited due to the phytotoxic properties of curcin [117–119]. Phorbol esters are highly toxic and carcinogenic, inducing leakage of plasma, tumors and tissue damage in humans and animals [120]. Curcin, has also been reported as stimuli for mucosal irritation and gastrointestinal hemagglutination in monogastric animals and humans [121]. The seed cake component phytic acid hinders the absorption of minerals in the intestines [122] while tannins reduce protein digestibility and stimulate liver damage [123]. Thus, the use of seed cake as an animal feed or fertilizer will only become feasible if the cake can be detoxified, which will not only aid commercial value to this by-product but would also diminish the environmental hazards caused by its inappropriate disposal.

In earlier studies, physical methods including heat-treatment, were able to detoxify curcin and tannins to a great extent, but not phorbol ester [124]. Among the toxins, phorbol esters have been recorded as heat resistant and the key toxic agents in *J. curcas* seed cake [116], where concentrations are reported to range from 0.21 to 0.47 mg/g [125]. To detoxify phorbol ester, chemical methods such as solvent extraction [17,21,111,126–131] and alkali treatments [132] have been developed and have shown up to 98% toxin reduction efficiency. However, a drawback of using chemical methods for detoxification are that the chemicals introduced in the process may result in other chemical residues. The progress on physical, chemical and biological detoxification processes for *J. curcas* seed cake was reviewed in Makkar et al. [133]. Efficiency and environmental safety are major advantages of biological detoxification over physical and chemical methods and recent research in detoxification has focused on eco-friendly biological detoxification using microorganism-mediated fermentation, and on toxin-free seed production using biotechnological tools. Biological detoxification is carried out using various strains of fungi and bacteria, several of which are efficient in toxin-reduction, as summarised in Table 2. The efficiency of Basidiomycete (Polyporales, Agaricales) and ascomycete (Saccharomycetales, Mucorales, Eurotiales and Hypocreales) fungi in detoxification of seed cake has been demonstrated in

Table 2
J. curcas seed cake detoxification experiments conducted by using biological agents.

Organism used	Experimental conditions for seed cake incubation with organisms	Targeted Toxin	Reduction (%)	Seed cake tested in animal feeding trial	Results of animal feeding trial	References
Bjerkandera adusta	Incubation at 28 °C for 30 days	Phorbol ester	91%	-	-	[198]
Phelbia rufa			97%	-	-	
Ganoderma resinaceum			No significant reduction	-	-	
Aspergillus niger	Incubation for 7 days followed by growth termination by oven drying at 70 °C	-	-	African dwarf goats were fed for 112 days	Seed cakes promoted tissue development in goats	[199]
Penicillium chrysogenum		-	-			
Trichoderma longibrachiatum		-	-			
Pseudomonas aeruginosa PseA	Incubation at 30 °C, pH 7.0 and relative humidity 65% for 9 days	Phorbol esters	100%	-	-	[135]
Lactobacillus plantarum *Propionibacterium*	Ensiling (60 days) by adding soluble carbohydrates in the cake and inoculant	Phorbol esters	47.40%	-	-	[136]
Pleurotus ostreatus	Incubated at 28 °C for 30 days	Phorbol ester Tanin Phytic acid	99% 46% 95%	Female alpine goats were fed for 60 days	No toxic effect on animals	[134]
Bacillus licheniformis *Bacillus subtilis*	Solid-state fermentation at 30 °C (for *B. subtilis*) and 37 °C (for *B. licheniformis*) for 7 days	Phorbol ester Phytic acid Trypsin inhibitor	Phorbol esters (32.8%), Phytic acid (57%), Trypsin inhibitor (1.2%)	-	-	[137]
Aspergillus oryzae	Solid-state fermentation for 12 days	Phorbol ester Phytic acid	Phorbol ester (57.8%) Phytic acid (92.9%)	-	-	[200]
Aspergillus terreus			Phorbol ester (57.8%) Phytic acid (92.1%)	-	-	
Aspergillus versicolor			Phorbol ester (68.6%) Phytic acid (64.6%)	-	-	
Aspergillus niger			Phorbol ester (69.8%) Phytic acid (96.5%)	-	-	
Penicillium miczynskii			Phorbol ester (56.6%) Phytic acid (89.8%)	-	-	
Cunninghamella echinulate			Phorbol ester (74.6%) Phytic acid (76.6%)	-	-	
Saccharomyces cerevisiae			Phorbol ester (63.8%) Phytic acid (91.2%)	-	-	
Rhizopus oryzae			Phorbol ester (62.6%) Phytic acid (73.5%)	-	-	
Pleurotus ostreatus	Incubation for 60 days	Phorbol esters	99%	-	-	[201]
Candida parapsilosis	Incubated at 30 °C for 24 h	Phorbol esters Curcin Trypsin inhibitor Saponin	100% 97.8% 64% 94.9%	-	-	[115]

animal feeding trials, with *Candida parapsilosis* showing up to 100% detoxification of phorbol ester in seed cake [115] followed by 99% reduction by *Pleurotus ostreatus* [134]. Among the bacteria studied, *Pseudomonas aeruginosa* showed 100% detoxification of phorbol ester in seed cake [135], compared to lower efficiencies by *Lactobacillus plantaru, Propionibacterium* (47.4% reduction) [137] *Bacillus licheniformis* and *Bacillus subtilis* (32.8% reduction) [137]. Although these results suggest strong potential for microbial-detoxification of *J. curcas* seed cake, more information is needed on the life cycle analysis of seed cake and of the environmental impact and economic feasibility for large-scale detoxification.

Genetic engineering approaches have also been explored to generate toxin free seeds of *J. curcas*, and provide a possibility to expand economic feasibility of *J. curcas* seed cake while minimizing the release of toxins into the environment [119,138]. The target of most studies has been the curcins, which are Type I ribosome inactivating proteins and are found in all accession of *J. curcas* including the edible Mexican *J. curcas* varieties which lack phorbol esters [139]. In *J. curcas*, the expression of the *Curcin 1* gene was mainly in endosperm, whereas *Curcin 2 A* was expressed in young leaves [139]. Patade et al. [119] conducted RNAi mediated constitutive silencing of *curcin 1* precursor mRNA in *J. curcas* using seed transformation. *Curcin 1* precursor transcript accumulation was compared in mechanically wounded leaves of transgenic and wildtype plants, as mechanical wounding leads to stress-induced expression of the *curcin 1* precursor, which is generally undetectable in leaves under non-stress conditions. The leaves from RNAi plants result showed 98% reduction in the curcin transcript compared to wild type plants. However, it was not reported if there is any reduction in curcin protein content in *J. curcas* seeds from this approach.

The finding that curcin expression increases during biotic and abiotic stresses raises the concern that “curcin-silenced” plants may be more susceptible to stress: Qin et al. [140] showed curcin expression was induced in response to fungal infection (*Pestalotia funerea*, *Curvularia lunata*, *Gibberelle zeae*) and abiotic stresses (drought and high temperature), while, ectopic expression of *Curcin 2 A* in transgenic tobacco increased disease tolerance against tobacco mosaic virus (TMV) and *Rhizoctonia solani* [141]. A solution to the above issue, by confining the gene silencing to the seeds, was demonstrated by Gu et al. [138]; using a marker-free transgenic *J. curcas* with curcin-deficient seeds this approach provided a solution to curcin toxicity as well as addressing the safety concerns of the marker genes. Gene silencing has also been used to engineer low phorbol ester *J. curcas* by seed specific silencing of two casbene synthase gene (*JcCASA163* and *JcCASD168*), reducing the precursor for phorbol esters [142]. Such gene silenced lines can be used as a parent in *J. curcas* breeding program to generate toxin free varieties. Simultaneous seed-specific silencing of multiple toxic-compound synthetic pathway genes may provide more suitable toxin free varieties.

2.4. Oil quality improvement

Seed oil content is the most economically important attribute of *J. curcas*. Plant oils are mainly composed of triacylglycerols (TAGs), molecules that comprise three fatty acid chains esterified to glycerol. To use as biodiesel, the seed oil is trans-esterified to fatty acid methyl esters [1]. We previously reported the oil content in *J. curcas* seed as around 23.70–46.60% [1]. As in other oleaginous plants, oil biosynthesis in *J. curcas* mainly occurs in the plastid and endoplasmic reticulum [143] (Fig. 3). Inside the plastids, de novo fatty acid synthesis starts with condensation of Acetyl-CoA to Malonyl-ACP, followed by a series of desaturation and hydrolysation reactions [reviewed in 144]. After synthesis, the free fatty acid is exported to the cytoplasm where it is converted into acyl-CoA. The acyl-CoA is transported to the endoplasmic reticulum and is acylated at the sn-1, sn-2, and sn-3 positions of glycerol-3-phosphate to produce TAG *via* an acyl-Co-A dependent pathway known as the Kennedy pathway [145]. An acyl-CoA-independent pathway also exists, where fatty acids are added to membrane lipids at the plastid envelope and/or in the endoplasmic reticulum *via* conversion of Diacylglycerol (DAG) to phosphatidylcholine (PC), an interchangeable process catalysed by choline phosphotransferase. PC acts as a donor of fatty acid for DAG to form TAG *via* Phospholipid: diacylglycerol acyltransferase (PDAT) [146]. After TAG biosynthesis is completed, oil droplets are accumulated as oil bodies (oleosome) in the cytoplasm.

There has been extensive progress in the exploration and characterization of genes associated with oil biosynthesis in *J. curcas*. Examples include: heteromeric subunit genes of ACCase (responsible for carboxylation of acetyl-CoA to produce malonyl-CoA) [147]; stearoyl-ACP (involved in the desaturation of stearoyl-ACP to Oleocyl-ACP [148]; two beta-ketoacyl-acyl carrier protein synthase genes *KASIII* [149] and *KAS II* [150] (associated with acyl chain elongation); acyl-ACP thioesterase gene (*FATB1*) (involved in the release of fatty acid) [151] and fatty acid desaturase genes *JcFAD2* and *JcFAD3* (involved in desaturation of oleic acid to linoleic acid and α-linolenic acid) [152]. *J. curcas* genes that have been associated with the Kennedy pathway include *GPAT* (Glycerol-3-phosphate acyltransferase) [153], *DGAT1* [154] and *DGAT2* (diglyceride acyltransferase) [155] and *PDAT* [156].

Fuel properties of *J. curcas* biodiesel reported in different geographical location are summarised in Table 3. Some important factors that are restricting the adoption of *J. curcas* biodiesel worldwide are its poor cold-flow properties, high viscosity, high oxidation rate and increased emission of nitrogen oxides (NOx) [reviewed in 157] relative to conventional fossil fuels. Cold flow properties and viscosity can be improved by decreasing saturated fatty acid levels, whereas, improving oxidative stability and reducing NO_X emission [reviewed in 158] requires a decrease in the proportions of unsaturated and poly-saturated fatty acids in the oil [reviewed in 159]. However, high unsaturation has a negative impact on the ignition quality of fuel [reviewed in 159] making it very challenging to find the ideal fatty acid profile that will deliver biodiesel with optimum parameters. A promising approach is to use oil with a high mono-unsaturated fatty acid content. We observed in our previous study that the fatty acid content in *J. curcas* mainly comprises the monosaturated fatty acid, oleic acid (41.48–46.1%) followed by the polysaturated fatty acid, linoleic acid (30.1–35.2%) [1]. Recent studies have reported increased oil content and modified fatty acid profiles from *J. curcas* based on the genetic manipulation of plants: Ectopic expression of *JcFATB1* (Fatty acyl-acyl carrier protein thioesterase) [151] and *JcKASIII* (β-ketoacyl-acyl carrier protein synthases II) [160] cDNA in Arabidopsis resulted in an increased content of palmitate and reduced levels of unsaturated fatty acids in seed oil. Ectopic expression of *JcDGAT1*-cDNA in Arabidopsis produced an increase in oil content by 30–40% and increase of linolenic acids in the TAG [154], whereas, expression of Arabidopsis *DGAT1* in *J. curcas* was reported to increase oleic acid content in TAG [81]. RNA Silencing approaches to reduce expression of oil biosynthesis genes have also showed some promise: silencing of *FAD2* (fatty acid desaturases 2) in *J. curcas* showed a hike in oleic acid content by more than 78% [161] while silencing of sugar-dependent 1 (*JcSDP1*), (an enzyme responsible for the first step of TAG degradation during seed germination) showed increase in TAG accumulation in *J. curcas* [162]. The availability of a whole genome sequence for *J. curcas* [143,163–166] provide more accessibility to oil biosynthetic genes known from their homologs in other plants, which can accelerate the development of trait-specific markers and the engineering of *J. curcas* varieties with desirable oil quality traits. In particular a clear characterization and evaluation of the functional application of genes of key enzymes involved in TAG synthesis are needed.

2.5. Advances in knowledge of abiotic stress biology of J. curcas

J. curcas has been reported to be effected by several types of abiotic stress such as high salinity, low temperature, drought and water logging, however its responses to such stresses are still poorly explored [34]. The impact of drought and salinity on *J. curcas* cultivation must be

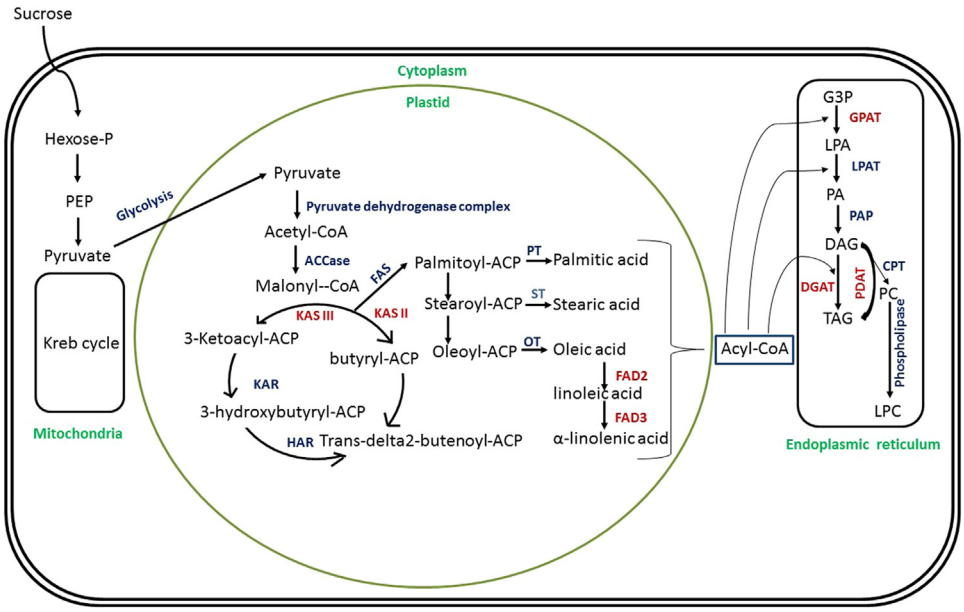

Fig. 3. Schematic representation of TAG biosynthesis ([143]; [226,227]). For simplicity, all possible reactions are not shown. The red ink represents the genes which are isolated and characterized in *J. curcas*. ACCase: acetyl-CoA carboxylase; PEP: Phosphoenolpyruvate; HAR: Hydroxyacyl-ACP dehydratase; KAR: β-ketoacyl-ACP reductase; KAS: β-ketoacyl-ACP synthase; PT: Palmitoyl Thioestrase; ST: Stearoyl Thioestrase; OT: Oleoyl Thioestrase; FAD: Fatty acid desaturases; G3P: Glycerol-3-phosphate; GPAT: Glycerol-3-phosphate acyltransferase; LPA: Lysophosphatidic acid; PA: Lysophosphatidic acid; LPAT: Lysophosphatidic acid acyltransferase; DAG: Diacylglycerol; TAG: Triacylglycerol; PDAT: Phosphatidyl choline transferase; PC: Phosphatidyl choline; CPT: Choline phosphotransferase; LPC: Lysophosphatidal choline.

known as a basis to standardize irrigation practices for large-scale cultivation. Gao et al. [167] analysed the impact of salinity stress on *J. curcas* seedlings under different NaCl concentrations (50–200 mM) and reported significant reduction in biomass compared to control seedlings. Salinity stress is associated with leaf yellowing, foliar damage and reduction in growth and biomass in *J. curcas*. However, leaf Na^+ (23.6–34.6 g kg DW^{-1}) and Cl^- concentrations (11.9 – 21 g kg DW^{-1}) were reported to be much higher than those detected in most glycophytes [168]. On the other hand, water stress conditions (drought) have a negative effect on seed productivity and oil content in field grown *J. curcas* [49]. Extreme drought conditions are reported to initiate leaf drop and limited root growth in *J. curcas* [19]. However, the higher level of proline synthesis recorded for drought-exposed *J. curcas* [127] may also help to explain the short drought-recovery periods (6–7 days) observed in terms of plant growth, stomatal structure and photochemical parameters [169,170]. At the other extreme, water-logging for a duration of 10 days resulted in reduced total biomass, leaf photosynthetic rate and carbohydrate content in both leaves and roots of *J. curcas* [171]. As most studies, have been carried out in controlled environments and with *J. curcas* seedlings, the extrapolation of data for field conditions still remains to be validated. In addition, no systematic assessment has been done to determine the impact of salinity stress on seed productivity. Therefore, further studies under field conditions and with mature *J. curcas* plants are needed to establish the impacts and to support the development of remedial measures.

At the molecular genetic level of abiotic stress biology, a number of transcription factors from *J. curcas*, have been associated with the regulation of stress-responsive genes [172,173]. The over-expression of *JcDREB,* a putative AP2/EREBP (ethylene responsive element binding protein) domain-containing transcription factor gene isolated from *J. curcas*, resulted in enhanced salt and freezing-stress tolerance in the model plant Arabidopsis [172]. Transgenic studies in *J. curcas* have also shown promise for salt and drought-stress tolerance [83,174]. The constitutive expression of a vacuolar Na + /H + antiporter gene from the halophyte plant *Salicornia brachiata*, in *J. curcas* provided increased salt tolerance (at 200 mM NaCl exposure) compared to wild type plants [83]. Genome-wide profiling of MYB family transcription factors in *J. curcas* led to the identification of 128 MYB genes, among which four subfamilies of domain repeats were observed including, 123 R2R3-MYB, four R1R2R3-MYBs and one 4R-MYB [173]. From these, over-expression of the nuclear localized *JcMYB2* in Arabidopsis, improved tolerance to salt and cold stress [173]. Tissue specific expression

Table 3
Properties of biodiesel from *J. curcas* from different geographical locations.

Geographical location	Oil content (%)	Viscosity (cst)	Density (g/cm^3)	Cloud point (°C)	Pour point (°C)	Flash point (°C)	Calorific value (MJ/kg)	References
Nicaragua (South America)	–	4.84	0.88	–	–	191	–	[202]
Faisalabad (Pakistan)	30–40	4.80 ± 0.17	0.88	10 ± 0.1	6 ± 0.2	188 ± 3.0	–	[203]
Sichuan (South west china)	–	4.06–5.13	0.82	–	–	164–166	–	[204]
Nigeria (Africa)	47.25	–	0.91	–	–	–	–	[205]
Malaysia	60	2.83	0.91	–	–	190.5	–	[206]
Nigeria	–	9.60	0.88	–	–	200	48.31	[207]
Brazil	31.6	4.02	0.88	–	− 5	117	40.31	[208]
China	–	3.6	–	1	–	153	–	[209]
Yunnan (South west China)	–	3.89	0.88	–	− 5	186	41.72	[210]
Nigeria	–	4.73	0. 86	–	–	184	–	[211]
Assam (India)	23.70–46.60	6.10–6.70	0.83–0.85	8	2	185	39.56–41.09	[1]
Malaysia	–	4.7227	0.86	5	3	182.5	39.827	[157]
Honduras (Central America)	–	2.7–3.0	–	3.8–4.3	–	–	–	[212]
Udaipur (India)	–	5.48 ± 0.06	0.86	–	–	–	39.47	[213]

profiles of *JcMYB2* in *J. curcas* showed it to be ubiquitously expressed throughout the plant, with higher transcript accumulation in roots [173]. Genome-wide analyses of salt [175], drought [176] and cold [177] -responsive transcriptomes of *J. curcas*, have identified numerous stress-responsive genes with potential for application in developing abiotic-stress tolerant varieties of the crop.

J. curcas is susceptible to water stress with poor performance under drought or water logging and research on transcription factors has also advanced knowledge in this area. *J. curcas* lines over-expressing Arabidopsis B subunit of the nuclear factor Y (*AtNF-YB1*) gene, showed enhanced content of the osmoprotectant, glycine betaine [174], although the impact of this on stress-tolerance was not studied. The adjustment of *J. curcas* roots against waterlogging-stress, explored at the transcriptome level *via* high-throughput RNA-sequencing (RNA-seq), identified the involvement of transcription factor families including AP2/ERF, MYB, and WRKY [178]. From differential gene expression data, it could be shown that water logging triggers responses in genes associated with hypoxia, anaerobic respiration, deterioration of cell wall biogenesis, carbohydrate production and growth in *J. curcas* [178] Since adverse climatic conditions are not only the norm for non-agricultural soils, but are expected to extend their range in the light of climate change, the findings on abiotic stress responses in *J. curcas* can provide new scopes for developing tolerant varieties, but also for other crops in the future.

2.6. Impact of biotic stress and measures to enhance cultivation of J. curcas

J. curcas seed oil contains compounds that are toxic to many microorganisms, insects and pests [179]. Despite the toxicity of the seeds, *J. curcas* plants are still susceptible to several pests and diseases, as a result of which, various measures have been explored for pest and disease control, some examples are shown in Table 4. The insect pest susceptibility of a plant species highly depends on the geographic location and environmental conditions, as these influence the presence of the pest or pathogen and the success of its infestation or infection. The major pests affecting *J. curcas* cultivars belong to the orders Lepidoptera, Hemiptera and Heteroptera. These pests have been reported to cause leaf mining, premature abortion of inflorescence and fruits and to account for 60–80% damage in large-scale plantation (Table 4). Spraying of insecticides can address these issues, however, may cause undesirable environmental issues including ground-water contamination and air pollution. The residues of chemical insecticides are toxic to humans and domestic animals [180] as well as to non-target and beneficial insects like honeybees, which play a very important role in *J. curcas* pollination and hence effect seed productivity [181]. A further complication is that poor management of insecticide application can result in the emergence of resistant insect biotypes [182].

Eco-friendly alternatives for pest and disease control have been explored, including use of biopesticides and other biocontrol measures, as these are biodegradable and have a reduced impact on the environment. Djimmy et al. [183] recently suggested controlling insect pests of *J. curcas* by exploring natural enemies of the insect: african shield bug (*Calidea panaethiopica)*, a polyphagous heteroptera of the Scutelleridae family, feeds on *J. curcas* flowers, fruit, and seeds thereby causing huge loss in seed production in the South-Sudanian Zone of Burkina Faso. Three parasites (*Trissolcus basalis*, Psixstriaticeps and Gryon sp.) were identified to infect the eggs of *C. panaethiopica* [183] with a peak of 43% parasitism during June to September. However, to exploit natural enemies associated with this insect pest, the biology, ecology, life cycle and host range of the predators should be thoroughly studied as well. Another viable alternative to chemical insecticides is the use of microbes *e.g. Bacillus thuringiensis* (*Bt*) [184]. Different strains of *Bt* are effective against specific Lepidopteran, Dipteran, Coleopteran, Hymenoptera, Homoptera, Orthoptera and Mallophaga insects as well as against nematodes and mites, which has led to their extensive use in crop protection [reviewed in 185]. However, two major limitations for using *Bt* spore formulations in crop protection are the limited use in wet climates or during rainy seasons, as formulations are easily washed away and the poor effectivity of sprayed spores against insect pests residing within plant tissues [186]. Transgenic crops expressing bacterial *Bt* toxins, have successfully addressed these challenges and are some of the most widely deployed and commercially successful plant biotech products. We previously reported transgenic *J. curcas* expressing the *Bt* toxin Cry1Ac protein which is toxic to the lepidopteran insect *Spodoptera litura* [187]. A marker-free transgenic line of *J. curcas* expressing hybrid Bt δ-endotoxin protein Cry1Ab/1Ac showed promising toxicity (80–100% mortality rate) towards the lepidopteran insect *Archips micaceanus* [188]. While these studies in *J. curcas* are at an early stage, the overwhelming success of the BT trait in other crops encourages us to believe that insect-resistant *J. curcas* lines are feasible and can increase seed yield in large-scale plantation while reducing the cost and environmentally detrimental effects of high pesticide use.

Fungal pathogens also can reduce *J. curcas* yields. While *J. curcas* leaf extract has shown inhibitory effect on mycelial growth of *Usarium graminearum, Pyricularia oryae, Phytophthora nicotianae* and *Phytophthora capsica* [189], *J. curcas* has been reported to be susceptible to several other fungal parasites, including *Fusariurn oxysporum* (wilting), *Rhizoctonia bataticola (*root rot), *Alternaria alternat* (leaf spot*)*, *Fusarium moniliforme* (root rot) and *Colletotrichum gloeosporioides (*Anthracnose) (Table 4). Spraying of fungicides such as carbendazim provided 100% inhibition of *Fusarium oxysporum* [190] and a combination of Bavistin and Vitavax at 50 ppm completely inhibited mycelial growth of *Rhizoctonia bataticola* (*R. bataticola*) [191]. However, as with insecticides, spraying of fungicides possesses environmental and health hazards, spurring research into alternate solutions. Kumar et al. [191] reported an integrated approach using plant extracts and pathogenic fungi together to reduce use of chemical fungicides: A combination of Bavistin (2 g/kg seed) with *Azadirachta indica* (neem extract) (20%) resulted in 67.3% inhibition of *R. bataticola* growth, while, a combination of *Trichoderma harzianum* (*T. harzianum) (*15 g mycelial mat /kg seed) and Bavistin showed 54.2% reduction in *R. bataticola* growth [191].

Other than insect and fungal parasites, *J. curcas* is also vulnerable to viruses, including various strains of *J. curcas* mosaic virus and Indian cassava mosaic virus (Table 4). There are several reports of virus-resistant plant lines which express viral coat protein or hairpin dsRNA with sequences homologous to viral genes [192]. Ye et al. [83], first reported development of transgenic *J. curcas* plants resistant to the geminivirus, Indian cassava mosaic virus (ICMV) *via* expression of a hairpin dsRNA of five ICMV DNA-A genes. Although initially laborious and expensive to generate, resistant transgenic lines can be used in as a parent in *J. curcas* breeding programs to rapidly multiply useful resistance traits. An alternate rapid development approach is to directly treat plants with dsRNA, which can be inexpensively made as a crude extract from bacteria expressing dsRNA with sequences homologous to viral mRNA [193,194]. The spraying should be done before the expected outbreaks of virus infection [194], such as when a vector insect infestation is first detected. This approach greatly depends on weather conditions, however, recent research on delivery of dsRNA loaded in clay nanoparticles (BioClay) showed the dsRNA to be stable and effective against crop viruses for an extended period of 20 days [195]. Thus, this approach could also be deployed for *J. curcas* in the future. The emerging research on biological approaches to *J. curcas* improvement are encouraging and attractive, as biological approaches are generally environmental safe. However, further research related to the specific pests and diseases of *J. curcas* is needed to develop solutions that are suitable for large scale deployment and are economically viable and stable.

3. Conclusions and recommendations

J. curcas has been shown to be a renewable source for sustainable

Table 4
Summary of biotic stress, area of occurrence and measures taken to control damage/infection in *J. curcas*.

Causal organism	Order	Damage/Symptom	Control suggested	Place reported	Reference
Insect					
Spodoptera litura	Lepidoptera	Devour the leaves, effect overall growth of the plant.	-	India (Madhya Pradesh)	[214]
Scutellera perplexa	Hemiptera	Fruit feeding led to premature fruit and seed abortion and reduction in yield.	-	India (Lucknow)	[18]
Maconellicoccus hirsutus	Hemiptera	Curling and contortion of leaves, stunted plant, deformed fruit, high abundance of insects can cause death of the tree.	-	India (Lucknow)	[215]
Stomphastis thraustica	Lepidoptera	Insect larvae feed on leaf blades by digging tunnels into them, leading to large brown patches on the leaves.	-	India (Secunderabad, Jhansi) Malaysia China (Panzhihua) Belgium (Lower Valley of the Senegal River)	[216,217] [218] [219] [220]
Pempelia morosalis	Lepidoptera	Fed on leaf and pollen causing damage to the inflorescences, stems and fruit.	-	Belgium (Lower Valley of the Senegal River)	[220]
Calidea panaethiopica	Heteroptera	Fed on the flowers and fruits of J. curcas. Attacked flowers turned dry dried and attacked fruits often have malformed or empty seeds.	-	Belgium (Lower Valley of the Senegal River) West Niger Burkina Faso	[220] [221] [183]
Fungi					
Fusarium oxysporum	Hypocreales	Wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system and stunted plants.	Spraying of carbendazim (200 µg/mL) showed 98% growth inhibition.	China (Hainan)	[190]
Rhizoctonia bataticola	Cantharellales	Root rot, symptoms of yellowing and dropping of leaves, dark brown lesions at collar region which spreads downwards in the plant.	Fungicides such as Bavistin and Vitavax at 50 ppm showed 100% inhibition of mycelial growth. Bavistin (2 g/ kg seed) + neem extract (20%) showed 67.3% reduction, *Trichoderma harzianum* (15 g per seed) + Bavistin and neem extract + T. harzianum showed 44.0% reduction	India (Haryana)	[191]
Alternaria alternata	Pleosporales	Amphigenous, irregular infection spots (1–2 cm) appear on both surfaces of leaf, which turns dark in the later stage.	-	India (Uttar Pradesh)	[222]
Fusarium moniliforme	Hypocreales	Yellowing of leaves leading to defoliation, drying of plants from tip downwards and ultimately death of the plant. On up- rooting the affected plants, browning/blackening of the roots was observed.	-	India	[223]
Colletotrichum gloeosporioides	Ascomycetes	Dark, water soaked lesions on stems, leaves or fruit.	-	Brazil and Cape Verde	[224]
Virus					
Mosaic virus	Begomovirus	Mild to severe mosaic, marked reduction in leaf size, rolling of leaf margins and puckering of the leaf surface. Chlorotic areas of irregular shapes were present between the secondary veins.	-	India	[225]
Jatropha mosaic virus	Geminivirus	Mild to severe mosaic, marked reduction in leaf size, rolling of leaf margins.	Transgenic *J. curcas* plants with resistance to ICMV *via* expression of a hairpin dsRNA with sequences homologous to five key ICMV DNA-A genes showed resistance.	Singapore	[83]
Indian cassava mosaic virus	Begomovirus	Yellow-green mosaic, curling, malformation and size reduction. In addition, infected plants also displayed other symptoms including shortened internodes, stunting of plant stature, fewer flowers which were partially or completely sterile.	-	India (Dharwad)	[10]

energy production, with good potential to address issues related to economic growth. Many of the valid concerns over various shortcomings of the crop arise from the requirement for production using marginal and contaminated soils so as to avoid competition for land for agricultural crop production. Despite of many challenges, the potential for *J. curcas* as biodiesel plant remains promising in the light of new knowledge and biotechnology. This review highlights the advancements from biological studies of *J. curcas* that are pertinent to application of this crop as a future sustainable biodiesel source. A summary of the main findings, from which our recommendations for further research have been made are as follows:

3.1. Improvement of seed yield of J. curcas via soil enrichment

Growing non-edible biodiesel crops in low nutrient and marginal soils offers a sustainable alternative approach to reclaim those lands while avoiding competition with food crops for land use. However, for cost-effective and profitable plantation, soil enrichments procedures are required. Although chemical fertilizers promote plant growth, their application leads to groundwater contamination, greenhouse gas emissions and disturbs the microflora and fauna of the soil. Successful alternative approaches are available *via* use of beneficial microbes such as AMF, endophytes and PGPR as biofertilizers. Application of AMF, endophytes and PGPRs led to enhanced seed production in *J. curcas* in low nutrient and stress conditions. However, the rhizosphere environment differs from that of internal plant tissues and also in different soil conditions. For example, the variations in light intensity, soil temperature, pH, nutrient availability, and the interaction with other organisms in the rhizosphere may influence performance. Therefore, it is recommended that indigenous species of AMF and PGPR are used in each locality. There are still several challenges such as optimising systems for low-cost mass production, and improving the viability of microbial inoculant during storage. In addition, use of elite cultivars of *J. curcas* with improved stress-tolerance will ameliorate some of the yield-reduction that results from poor nutrition and contaminants in marginal soils.

3.2. Manipulation of inflorescence development to enhance seed yield

Plants producing inflorescences with an increased female flower ratio is a highly desirable trait for biodiesel crops. While exogenous application of plant growth regulators such as gibberellins, cytokinin and thidiazuron have shown substantial increase in female flowers, the process is labour intensive and success is weather-dependent. Recent advancement in genomics and transcriptomics, provides more options for improvement in female flower numbers in *J. curcas via* manipulation of plant growth regulator biosynthesis pathways or key sex-related genes responsible for sex differentiation at the developmental biology level. The over expression of flowering locus T homolog (*JcFT*) [97] and LEAFY (*JcLFY*) [98] showed promise in this respect and produced plants with higher numbers of female flowers. Average seed yield in *J. curcas* can be improved by providing suitable pollination services to optimise fruit set *via* cross pollination using honey bee species that have been recorded as the most efficient pollinators. Therefore, the strategical establishment of native bee hubs inside plantation areas is recommended.

3.3. Detoxification of seed cake to aid economic value of J. curcas plantations

Seed cake detoxification using fungal and bacterial strain has shown promising results. The fungi *Candida parapsilosis* [115] and *Pleurotus ostreatus* [134] and the bacteria *Psesudomonas aeruginosa* [135] were observed as the most efficient bioagents for detoxification. Following detoxification, seed cakes can be used as a component of biofertilizers and animal feed. However, for profitable industrial implementation of seed cakes life cycle analysis, environmental impact assessment and cost analysis are needed. In addition, the toxin-free seeds from genetically modified *J. curcas* lines can aid in economic feasibility by reducing the processing costs for toxin-free seed cakes for fertilizers and animal feed.

3.4. Modification of fatty acid composition for improvement of biodiesel properties

Oil high in mono-saturated fatty acid and low in both saturated and polysaturated fatty acid is always desirable as biodiesel feedstock to address issues such as cold flow properties, oxidative stability and NOx emissions. Some progress has been achieved through the manipulation of fatty acid biosynthesis genes, such as reduced poly-unsaturated fatty acid levels in model plants through the over-expression of fatty acyl-acyl carrier protein thioesterase (*JcFATB1*) [151] and β-ketoacyl-acyl carrier protein synthases II (*JcKASIII)* [160]. Increased monosaturated fatty acid (oleic acid, 18:1) content and the reduction in both saturated and polysaturated fatty acid in Jatropha seed oil were also demonstrated after over-expression of Arabidopsis diglyceride acyltransferase *DGAT1* [81] and silencing of fatty acid desaturase 2 (*FAD2*) [157] in *J. curcas*. Further improvement in cold flow properties could be achieved by increasing the short chain mono-saturated (palmitoleic acid, 16:1) and saturated fatty acid content of *J. curcas* seed oil *via* silencing fatty acid desaturases responsible for conversion of long chain saturated (*e.g.* 18:2, 18:3) or mon-unsaturated (*e.g.* 18:1, 20:1) fatty acid. To date, relatively few genes have been studied and further functional characterization, in particular for TAG biosynthesis genes, can advance this approach to Jatropha improvement. Recent publication of the whole genome sequence for *J. curcas* can facilitate both genetic engineering and molecular breeding approaches to develop *J. curcas* lines with desired oil quality traits.

3.5. Improving abiotic stress tolerance

Abiotic stress factors such as salt, drought and waterlogging limit seed productivity of *J. curcas*. The early approach to combat abiotic stress was to develop stress-tolerant cultivars using conventional breeding. However, no modified stress-tolerant cultivars have been reported *via* breeding as the process is not only labour intensive but also time consuming for a perennial crop such as *J. curcas.* Early research using a genetic modification approach to overexpress a vacuolar Na + /H+ antiporter from *Salicornia brachiata* in *J. curcas* showed promise, including a *J. curcas* variety with increased tolerance to salt stress, however field validation of novel hybrids will be needed before commercial viability is assured. Meanwhile, stress-responsive gene discovery is providing several promising target genes to test (*e.g.* the transcription factors *JcDREB, JcMYB, AP2/ERF* and *WRKY*) and it is anticipated that, as for other crops, new varieties that can produce good yields on marginalised soils will emerge in time, if given the needed research support for development and evaluation.

3.6. Improving biotic stress tolerance

Despite the toxins found in the seed, Jatropha is susceptible to numerous pests and diseases. Two potentially complementary biological approaches have promise here, which are biopesticide/biological control of pests, and genetic improvement of resistance traits in the plant, leveraging new knowledge on pest and disease responsive genes in *J. curcas* as well as from other crops. Another potential approach is to exploit natural parasitic relationships among insect pests, which has been reported for the heteropteran insect *Calidea panaethiopica*. Knowledge on biology, ecology, life cycle and host range of the predators should be obtained prior to application. Development of insect resistance is another stable approach to control insect manifestation, by deploying suitable candidate genes. Other biotic stresses such as fungal

attack can be diminished by application of combination of pathogenic fungus and plant extracts. Viral attacks can be suppressed by spraying dsRNA loaded in bio-clay containing homologous sequences of viral mRNA or developing virus resistance transgenic *J. curcas*. However, both gene silencing approaches have pros and cons, direct treatment with dsRNA is rapid but able to give protection only up to 20 days after each spraying, while the development of transgenic resistant plant lines takes longer but can provide more stable protection of the plant throughout the lifetime.

4. Future prospects

Based on the progress in biological research together with newly emerging technology, there are excellent prospects to advance *J. curcas* as a bioenergy crop for use on marginal soils. Firstly, the use of bioagents including microbes as biofertilizers and biopesticides has shown promising results for enriching soil fertility and in providing protection against biotic and abiotic stresses, with the additional benefits of reduced input cost, improved environmental safety and target-specificity. The exploration of the molecular basis of host interactions with beneficial microbes will further facilitate establishment of a stable eco-friendly and sustainable *J. curcas* plantation, avoiding or at least reducing the use of chemical fertilizers, insecticides or pesticides.

Secondly, the availability of the whole genome sequence, efficient transformation systems and genetically modified lines of *J. curcas*, offer exciting opportunities to develop elite commercial cultivars with high yield along with tolerance to biotic and abiotic stresses. Early studies have shown that both over-expression and gene silencing methods can address the various limitations of this crop. However, since the deployment of genetically modified crops, still faces issues of public acceptance in several countries, alternative approaches can be explored such as direct application of dsRNA or gene editing with site specific nucleases [196]. The emergence of highly precise genome editing techniques which involve specific alterations of single or multiple genetic loci, has been effective as a novel targeted approach to crop mutagenesis which in some legislations is not regulated as a genetic modification [reviewed in 197]. It is attractive to think that site-specific nucleases such as clustered regularly interspaced short palindromic repeat (CRISPR)-associated endonuclease 9 (CRISPR/cas9) that have shown successful results in plant improvement [198] can also be used in *J. curcas*. While the efficacy of these approaches in genetic improvement of *J. curcas* has not been demonstrated so far, it is feasible that site-directed mutagenesis of precursor genes of curcin and phorbol ester could generate varieties of *J. curcas* with toxin-free seed. Similarly, gene silencing and editing can be used to develop elite cultivars with many of the currently lacking traits, and also provide the possibility of combining abiotic and biotic stress resistance, improved oil quality for biodiesel use and toxin-free seed cake as a commercially valuable by-product. A combinatorial approach using elite lines from conventional breeding programs, complemented by and crossed with those produced *via* biotechnological tools can expedite crop improvement. Taken together biological information and biotechnological approaches can help to bridge the gaps in productivity for *J. curcas*.

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